

In re Appln. of RATLEDGE et al.  
Application No. 10/030,700

### CLAIM AMENDMENTS

Claims 1-33 (Cancelled).

34. (Currently Amended) A method of producing an oil including docosahexaenoic acid (DHA) with a strain of *Cryptecodinium cohnii* comprising:

culturing a strain of *Cryptecodinium cohnii* in a nutrient medium containing ~~a compound selected from the group consisting of~~ acetic acid and/or acetate ions, ~~the acetic acid and/or acetate ions comprising~~ Cryptecodinium cohnii using the compound as the primary carbon source consumed by the *Cryptecodinium cohnii* to synthesize the DHA, wherein the culturing process parameters are controlled in a manner that results in the absence of a stationary phase during the culturing process, and recovering oil including DHA from the strain of *Cryptecodinium cohnii*.

Claims 35-36 (Cancelled).

37. (Currently Amended) The method according to claim 34, wherein the consumption of the acetic acid and/or acetate ions by the *Cryptecodinium cohnii* ~~using the compound~~ as the primary carbon source causes an increase in pH of the nutrient medium and the method further includes monitoring the pH of the nutrient medium and adding ~~more compound~~ acetic acid and/or acetate ions to the nutrient medium in response to an increase in the pH of the nutrient medium.

38. (Currently Amended) The method according to claim 37, wherein the adding more compound to the nutrient medium comprises adding more compound ~~is in an amount effective~~ to maintain the pH of the nutrient medium substantially at a value of between about 5 and about 8.

39. (Currently Amended) The method according to claim 38, wherein the adding more compound to the nutrient medium comprises adding more compound ~~is in an amount effective~~ to maintain the pH of the nutrient medium at about 6.5.

40. (Currently Amended) The method according to claim 37, wherein the pH of the nutrient medium is monitored by means communicating with a control device, and wherein the control device controls the adding more compound to the nutrient medium.

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Claims 41-42 (Cancelled).

43. (Currently Amended) The method according to claim 37, wherein ~~the adding more compound to the nutrient medium comprises adding more compound to the nutrient medium~~ is in a mixture including an organic acid.

44. (Currently Amended) The method according to claim 37, wherein ~~the adding more compound to the nutrient medium comprises adding more compound to the nutrient medium~~ is in a mixture including a lipid.

45. (Currently Amended) The method according to claim 37, wherein ~~adding more compound to the nutrient medium comprises adding more compound which is~~ the acetic acid and/or acetate ions are supplied from a waste product from an industrial process.

46. (Currently Amended) The method according to claim 37, wherein ~~the adding more compound to the nutrient medium comprises adding more compound to the nutrient medium~~ is in a mixture including a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

47. (Currently Amended) The method according to claim 34, wherein ~~the nutrient medium containing the compound comprises a second nutrient medium and prior to culturing the strain of *Cryptocodinium cohnii* in the second nutrient medium, the strain of *Cryptocodinium cohnii* is cultured~~ prior to culturing the strain of *Cryptocodinium cohnii* in acetic acid and/or acetate ions, an inoculum containing the strain of *Cryptocodinium cohnii* is prepared by culturing in a first nutrient medium containing glucose.

Claim 48 (Cancelled).

49. (Currently Amended) The method according to claim ~~48~~ 34, wherein ~~the organic nitrogen source is the nutrient medium contains yeast extract and the~~ in an initial concentration of the yeast extract in the nutrient medium is greater than 7.5 g/l.

50. (Previously Presented) The method according to claim 49, wherein the initial concentration of yeast extract in the nutrient medium is 10 g/l.

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Claims 51-73 (Cancelled).

74. (Previously Presented) The method of claim 34, wherein culturing the strain of *Cryptocodinium cohnii* is performed as a continuous or semi-continuous process.

Claim 75 (Cancelled).

76. (Currently Amended) The method according to claim 34 further comprising purifying the oil recovered from the strain of *Cryptocodinium cohnii* to increase the docosahexaenoic acid content of the oil.

Claim 77 (Cancelled).

78. (Currently Amended) The method according to claim 34 wherein the initial concentration of the compound acetic acid and/or acetate ions in the culture is between 4 and 16 g/l.

79. (Currently Amended) The method according to claim 78, wherein the initial concentration of the compound acetic acid and/or acetate ions is about 8 g/l.

Claim 80 (Cancelled).

81. (Currently Amended) The method according to claim 34, wherein the percent docosahexaenoic acid in the oil recovered from the strain of *Cryptocodinium cohnii* is at least ~~29.3~~ 28.9.

82. (Currently Amended) The method according to claim 34, wherein ~~during the~~ after culturing ~~process for 72 hours~~, the total concentration of docosahexaenoic acid synthesized by the strain of *Cryptocodintum cohnii* ~~also to~~ is at least 0.9 grams per liter of nutrient medium.

Claims 83-86 (Cancelled).

**CLAIM AMENDMENTS**

Claims 1-33 (Cancelled).

34. (Currently Amended) A method of producing an oil including docosahexaenoic acid (DHA) with a strain of *Crypthecodinium cohnii* comprising:

culturing a strain of *Crypthecodinium cohnii* in a nutrient medium containing acetic acid and/or acetate ions, the acetic acid and/or acetate ions comprising the primary carbon source consumed by the *Crypthecodinium cohnii* to synthesize the DHA, wherein the culturing process parameters are controlled in a manner that results in the absence of a stationary phase during the culturing process, and

recovering oil including DHA from the strain of *Crypthecodinium cohnii*.

Claims 35-36 (Cancelled).

37. (Currently Amended) The method according to claim 34, wherein the consumption of the acetic acid and/or acetate ions by the *Crypthecodinium cohnii* as the primary carbon source causes an increase in pH of the nutrient medium and the method further includes monitoring the pH of the nutrient medium and adding more acetic acid and/or acetate ions to the nutrient medium in response to an increase in the pH of the nutrient medium.

38. (Currently Amended) The method according to claim 37, wherein the adding is in an amount effective to maintain the pH of the nutrient medium substantially at a value of between about 5 and about 8.

39. (Currently Amended) The method according to claim 38, wherein the adding is in an amount effective to maintain the pH of the nutrient medium at about 6.5.

40. (Currently Amended) The method according to claim 37, wherein the pH of the nutrient medium is monitored by means communicating with a control device, and wherein the control device controls the adding.

Claims 41-42 (Cancelled).

43. (Currently Amended) The method according to claim 37, wherein the adding is in a mixture including an organic acid.

44. (Currently Amended) The method according to claim 37 , wherein the adding is in a mixture including a lipid.
45. (Currently Amended) The method according to claim 37, wherein the acetic acid and/or acetate ions are supplied from a waste product from an industrial process.
46. (Currently Amended) The method according to claim 37 , wherein the adding is in a mixture including a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.
47. (Currently Amended) The method according to claim 34, wherein prior to culturing the strain of *Cryptocodinium cohnii* in acetic acid and/or acetate ions, an inoculum containing the strain of *Cryptocodinium cohnii* is prepared by culturing in a nutrient medium containing glucose.

Claim 48 (Cancelled).

49. (Currently Amended) The method according to claim 34, wherein the nutrient medium contains yeast extract in an initial concentration greater than 7.5 g/l.
50. (Previously Presented) The method according to claim 49, wherein the initial concentration of yeast extract in the nutrient medium is 10 g/l.

Claims 51-73 (Cancelled).

74. (Previously Presented) The method of claim 34, wherein culturing the strain of *Cryptocodinium cohnii* is performed as a continuous or semi-continuous process.

Claim 75 (Cancelled).

76. (Currently Amended) The method according to claim 34 further comprising purifying the docosahexaenoic acid.

Claim 77 (Cancelled).

78. (Currently Amended) The method according to claim 34 wherein the initial concentration of the acetic acid and/or acetate ions in the culture is between 4 and 16 g/l.

79. (Currently Amended) The method according to claim 78, wherein the initial concentration of the acetic acid and/or acetate ions is about 8 g/l.

Claim 80 (Cancelled).

81. (Currently Amended) The method according to claim 34, wherein the percent docosahexaenoic acid in the oil recovered from the strain of *Cryptocodinium cohnii* is at least 28.9.

82. (Currently Amended) The method according to claim 34, wherein after culturing for 72 hours, the total concentration of docosahexaenoic acid synthesized by the strain of *Cryptocodinium cohnii* is at least 0.9 grams per liter of nutrient medium.

Claims 83-86 (Cancelled).